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Influence of dual salt on the pretreatment of sugarcane bagasse with hydrogen peroxide for bioethanol production

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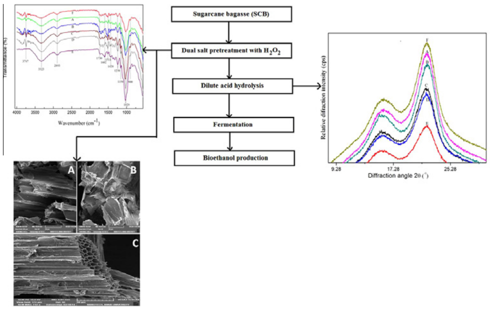
HIGHLIGHTS

- Influence of dual salt on the pretreatment of SCB with H_2O_2 was carried out.
- Better performance was observed using H_2O_2 with $MnSO_4{\cdot}H_2O$ and ZnO.
- The inhibitors formation was found to be less.
- The maximum theoretical ethanol yield of 84.32% was achieved during fermentation.

GRAPHICAL ABSTRACT



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ABSTRACT

This study presents the influence of different dual salts on the pretreatment of sugarcane bagasse (SCB) with H_2O_2 for the production of bioethanol. The results showed better cellulose recovery and delignification for $MnSO_4{\cdot}H_2O$ and ZnO system. At optimized conditions (1% H_2O_2, 1 g SCB, 30 min, 100 °C and 0.5:100 g/mL), the maximum cellulose recovery of 93.42 ± 1.74%, and delignification of 74.18 ± 1.51% were obtained. The SCB was subjected to dilute acid hydrolysis, and under optimized conditions, the maximum glucose, xylose and arabinose concentrations obtained were 15.26 ± 0.37 g/L, 11.62 ± 0.21 g/L and 3.58 ± 0.17 g/L, respectively. The results of SEM, FTIR and XRD analysis suggested that the proposed method effectively removed lignin and disrupted the crystal structure of cellulose for a better hydrolytic process. The fermentation of hydrolyzate with *Saccharomyces cerevisiae* showed maximum bioethanol production of 13.1 g/L at the end of 72 h and bioethanol yield of 0.184 g/g SCB.

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1. Introduction

The depletion of fossil fuel reserves and increase in greenhouse gas emissions necessitated the production of economically feasible alternative fuels from biomass. Lignocellulosic biomass (LCB) is an attractive feedstock for the production of second generation biofuels since they are cheap and abundant in nature [1]. LCBs including corn stover, rice straw, sugarcane bagasse (SCB), garden wastes and forest residues, were successfully used for ethanol production.

SCB is mainly composed of cellulose, hemicellulose and lignin. The conversion of complex cellulose and hemicellulose present in SCB into fermentable sugar poses a technical and economic challenge. The crystalline nature of cellulose and the strong protective layer around the lignin and hemicellulose hinder the breaking of β-(1, 4) – glycosidic bonds of cellulose by acid/enzyme catalysts. In order to facilitate the hydrolytic reaction, a variety of pretreatment methods including alkali pretreatment, acid hydrolysis, steam explosion, liquid hot water process, wet oxidation, ammonia fiber explosion (AFEX), biological pretreatment and pretreatment with

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hydrogen peroxide have been developed [2]. The pretreatment process disrupts the crystalline structure and reduces the degree of polymerization of cellulosic region. This makes cellulose and hemicellulose more suitable for hydrolytic process, which converts these polymers to fermentable sugars [3]. It should be noted that few pretreatment processes require high pressure, high temperature and chemicals like acid or alkali [4].

The performance of pretreatment can be improved by the addition of inorganic salts [5] and the main advantage of using inorganic salts is that it is recyclable and less corrosive than inorganic acids [6]; also it alters the structure and greatly affects the composition of biomass [5]. The combined effect of alkaline hydrogen peroxide with metal ions produces hydroxyl radicals and superoxide ions, which decompose the lignin [7]. The effective formation of phenoxy radicals is due to the action of hydroxyl radical and metal ions [8]. The usage of inorganic salts expected to increase the hydrolysis rate of pentose and hexose sugar and this result in higher sugar yields [9,10].

The aim of the present study was to maximize the cellulose recovery and delignification besides reducing the pretreatment time and inhibitors formation. The effect of various dual salts on the pretreatment of sugarcane bagasse with hydrogen peroxide was studied and the influence of operating parameters such as H_2O_2 concentration, SCB dosage, reaction rime, temperature and molar ratio of metal salts to H_2O_2 was also studied. The pretreatment was performed at moderately rigorous conditions in order to reduce the formation of inhibitory products.

2. Materials and methods

2.1. Materials

Sugarcane bagasse (SCB) was collected from the local sugarcane processing unit located at Chennai, Tamil Nadu. SCB was washed thoroughly with distilled water to remove the soluble sugars, and kept in a hot air oven at 60 °C for 24 h. The dried SCB was ground and screened to a particle size, below 1 mm (>18 mesh size, ASTM Standard) using a sieve shaker. The sieved biomass was stored in an air tight polyethylene bag, at room temperature for further use. The chemicals such as 3, 5-dinitrosalicylic acid, manganese sulfate, acetone were purchased from Sisco Research Laboratory Pvt., Ltd., Mumbai, India, sulfuric acid was purchased from Thermo Fischer Scientific India, Ltd., Mumbai, India, 30% hydrogen peroxide was purchased from Merck Specialties Private Ltd., Mumbai, India, and zinc oxide was purchased from Sigma–Aldrich chemicals Pvt., Ltd., Bangalore, India. The chemicals used in this investigation were analytical grade and used as such without any further treatment.

2.2. Pretreatment

The SCB was pretreated using hydrogen peroxide in the presence of dual salt. Metal salts such as manganese sulfate monohydrate ($MnSO_4{\cdot}H_2O$), zinc oxide (ZnO), ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), cobalt chloride ($CoCl_2$), and titanium dioxide (TiO_2) were employed. The influence of operating parameters, such as H_2O_2 concentration (0.25, 0.50, 1 and 2% v/v), SCB dosage (1, 2, 3 and 4 g), reaction time (15, 30, 45 and 60 min), temperature (25, 50, 75 and 100 °C) and molar ratio of metal salts to H_2O_2 (0.5:100, 1:100, 2:100 and 4:100 g/mL) were investigated. The pretreated SCB was filtered, washed several times with distilled water, hot water and finally with acetone to remove metal salts. The filtered residue was dried at 60 °C until a constant weight was observed, and then it was subjected to dilute acid hydrolysis. The % cellulose recovery in the solid content was calculated using the following equation.

$$\%\ \text{Cellulose recovery} = \frac{R_{\text{PT-SCB}}}{R_{\text{SCB}}} \times 100 \tag{1}$$

where R_{SCB} is the amount of cellulose in native SCB and $R_{\text{PT-SCB}}$ is the amount of cellulose in pretreated SCB expressed in (g/g).

The % delignification was calculated using the following equation:

$$\%\ \text{Delignification} = \left(\frac{D_{\text{SCB}} - D_{\text{PT-SCB}}}{D_{\text{SCB}}}\right) \times 100 \tag{2}$$

where D_{SCB} is the amount of lignin present in the native SCB, and $D_{\text{PT-SCB}}$ is the amount of lignin in the pretreated SCB, measured in (g/g).

2.3. Hydroxyl radicals measurement

The hydroxyl radicals formed during the pretreatment of SCB using dual salt with H_2O_2, played a vital role in removing lignin. In the present study, hydroxyl radicals formed were measured by Fricke dosimetry [11]. The Fricke solution was prepared, using 1 mM $FeSO_4$, 0.4 M H_2SO_4 and 1 mM NaCl. The amount of hydroxyl radicals present was estimated based on the oxidation of Fe^{2+} to Fe^{3+}. Initially, Fricke solution was added with SCB, H_2O_2 and dual salt. Then, the reaction was carried out as per the pretreatment conditions. The samples were withdrawn at regular time intervals and Fe^{3+} formed was determined by observing the absorbance at 304 nm using Elico double beam SL210 UV–Visible spectrophotometer. The number of hydroxyl radicals formed in the Fricke solution is theoretically equal to one quarter the amount of Fe^{3+} produced [12]. The concentration of H_2O_2 was indirectly measured by the iodine method [13]. About 2 mL of liquid sample was mixed with 0.75 mL of iodine indicator (0.06 M sodium hydroxide, 0.2 mM ammonium molybdate and 0.4 M potassium iodide) and 1 mL of 0.1 M potassium biphthalate. The absorbance of the treated sample was measured at 351 nm in a UV–Visible spectrophotometer.

2.4. Dilute acid hydrolysis

Dilute acid hydrolysis was carried out in an Erlenmeyer flask (250 mL) containing SCB with maximum cellulose content. About 1 g of pretreated SCB was suspended in different concentrations (0.25, 0.5, 1 and 2% v/v) of sulfuric acid solutions and experiments were carried out at 121 °C and at a constant solid-to-liquid ratio of 1:20 g/mL for 15 min. After the hydrolysis, the mixture was cooled to room temperature. Then, the samples obtained were centrifuged at 8000 rpm for 10 min and the supernatant obtained was subjected to glucose, xylose, arabinose, acetic acid and furfural analysis [14]. The optimum concentration was selected based on the sugar yield. The hydrolyzate obtained was neutralized using sodium hydroxide (2 N) to obtain the neutral pH solution for fermentation. All the experiments were carried out in triplicate and the average values were reported. The catalytic efficiency [E] of dilute acid hydrolysis was calculated, using the following equation [15]:

$$[E] = \frac{\sum S}{1 + \sum I} \tag{3}$$

where $\sum S$ is the sum of the sugar concentrations in the hydrolyzate (glucose, xylose and arabinose) and $\sum I$ is the sum of the inhibitor concentration in the hydrolyzate (acetic acid and furfural).

2.5. Fermentation

The hydrolyzate obtained was fermented using *Saccharomyces cerevisiae.* About 100 mL of the hydrolyzate was placed in a 250 mL Erlenmayer flask and it was supplemented with synthetic medium containing peptone (5 g/L), yeast extract (3 g/L), $CaCl_2$ (0.025 g/L), $MgCl_2$ (0.025 g/L) and KH_2PO_4 (25 g/L). The contents were sterilized at 121 °C, allowed to cool and then inoculated with 24 h old seed culture of *S. cerevisiae* maintained at 30 °C. The samples withdrawn at regular time intervals were subjected to ethanol analysis.

2.6. Analytical methods

Cellulose, hemicellulose, lignin and ash content were estimated by detergent extraction method [16]. About 1 g of dried SCB (pretreated) was refluxed in a 250 mL round bottom flask containing neutral detergent solution, 2 mL of decahydronaphthalene and 0.5 g of sodium sulfite. The reaction was carried out for 1 h and then, the contents were filtered in a crucible and washed thrice with hot distilled water. Finally, the contents were washed with acetone, dried overnight at 100 °C and weighed to obtain the value of NDF.

The NDF sample was placed in a 250 mL round bottom flask containing 100 mL acid detergent solution and the contents were refluxed for 60 min from the onset of boiling. After the reaction, the contents were filtered, washed thrice with hot distilled water and finally washed with acetone. The sample was dried overnight at 100 °C and weighed hot. The difference between NDF and acid detergent residue gave the amount of hemicelluloses present. The acid detergent residue was placed in a crucible and half filled with 72% sulfuric acid. The contents were stirred using glass rods to wet the contents completely. After 3 h, sulfuric acid was removed by suction and the residue was washed thoroughly with hot distilled water. The crucible was dried overnight at 100 °C and weighed. Cellulose was determined as the loss in weight from acid detergent residue. Finally, the residue was ashed in the crucible for 3 h at 550 °C and kept overnight to cool. The lignin content was calculated as the loss in weight upon ashing.

The reducing sugars produced during the dilute acid hydrolysis were determined by 3,5-dinitrosalicyclic acid (DNS) method [17]. Glucose, xylose, arabinose, acetic acid and ethanol were determined using high performance liquid chromatography (Agilent 1260 with RI detector and Hi-Plex H column (300 × 7.7 mm)) operated with 5 mM H_2SO_4 as mobile-phase at a flow rate of 0.6 mL/min [18]. Furfural was detected using UV chromatograms at 280 nm. The changes in the functional groups of SCB before and after the pretreatment process were analyzed using Fourier-transform infrared spectroscopy (FTIR) (Perkin-Elmer FTIR Spectrophotometer 2000 series) with the detector at 4 cm^{-1} resolution and 25 scan per sample. The pellets were prepared using 2 mg of dried and pretreated biomass and 200 mg KBr. The spectrum was recorded between 4000 and 550 cm^{-1}. The crystalline nature of SCB, before and after the hydrolysis was examined using X-ray diffractometer (XRD, PANalytical X'Pert PRO) with the applied potential of 40 kV and current 30 mA. The samples were scanned in the range of $2\theta = 5–35°$ with a step size of 0.05° and the crystallinity index (CrI) was determined using the equation given below [19].

$$\mathrm{CrI}\ (\%) = \left[\frac{I_{\mathrm{Crystalline}} - I_{\mathrm{Amorphous}}}{I_{\mathrm{Crystalline}}}\right] \times 100 \quad (4)$$

where $I_{\mathrm{Crystalline}}$ = maximum diffraction intensity at 21° and $I_{\mathrm{Amorphous}}$ = diffraction intensity at 18.8°, 2θ degrees.

The structural and surface morphology of raw, pretreated and dilute acid hydrolyzed SCB (dried biomass passing through 40 mesh sieve) were analyzed using VEGA 3 TESCAN (Czech Republic). The native and pretreated SCB were mounted with double sided carbon tape on brass sample stubs and sputter coated with a gold film using EMITECH SC 7620. This assembly was kept in a vacuum desiccator until the analysis. Sample images were taken at 10 kV accelerating voltage and the SCB samples were examined at a magnification ranging from 500× to 1400×.

3. Results and discussion

3.1. Effect of H_2O_2 with dual salts

The composition SCB used in this study was 38.0 ± 0.4% cellulose, 32.0 ± 0.2% hemicellulose, 27.0 ± 0.1% lignin, 2.3 ± 0.3% ash and 0.7 ± 0.3% others. SCB was pretreated using H_2O_2 with various combinations of metallic salts and their influence on cellulose recovery and delignification is shown in Fig. 1. The % cellulose, hemicellulose, lignin and ash were calculated on dry weight basis. The pretreatment with H_2O_2 in the presence of $MnSO_4{\cdot}H_2O$ showed 72.61% cellulose recovery and 18.48% delignification. The treatment of H_2O_2 with manganese salts hardly solubilized hemicellulosic and lignin fractions as the weight of pretreated SCB showed little loss in dry matter. The maximum cellulose recovery (83.92 ± 2.4%) and delignification (58.37 ± 1.9%) were obtained with $MnSO_4{\cdot}H_2O$ and ZnO. The presence of manganese salts known to promote the degradation of lignin due to its high catalytic activity [8]. On the other hand, H_2O_2 reacts with various carbonyl structures of lignin such as quinones and cinnamaldehyde besides very effective attack on phenols [8]. The production of more cations increases the hydroxyl radicals ($^{\cdot}OH$) formation and this facilitates the oxidative depolymerization of complex phenolic structures of lignin. Next to this combination, the order for the maximum recovery of cellulose was $MnSO_4{\cdot}H_2O$ and $CoCl_2$, $MnSO_4$ and $(NH_4)_6Mo_7O_{24}$, $MnSO_4{\cdot}H_2O$ and $FeSO_4{\cdot}7H_2O$, and $MnSO_4{\cdot}H_2O$ and TiO_2. For $MnSO_4{\cdot}H_2O$ + ZnO and $MnSO_4{\cdot}H_2O + CoCl_2$, the % cellulose recovery observed was almost similar. In the case of delignification, the order was $MnSO_4{\cdot}H_2O + ZnO > MnSO_4{\cdot}H_2O + FeSO_4{\cdot}7H_2O > MnSO_4{\cdot}H_2O + CoCl_2 > MnSO_4{\cdot}H_2O + (NH_4)_6Mo_7O_{24}) > MnSO_4{\cdot}H_2O + TiO_2$. The results showed less recovery of cellulose and delignification for $MnSO_4{\cdot}H_2O$ and TiO_2 and this may be due to the lesser $^{\cdot}OH$ formation. To confirm this, hydroxyl radicals

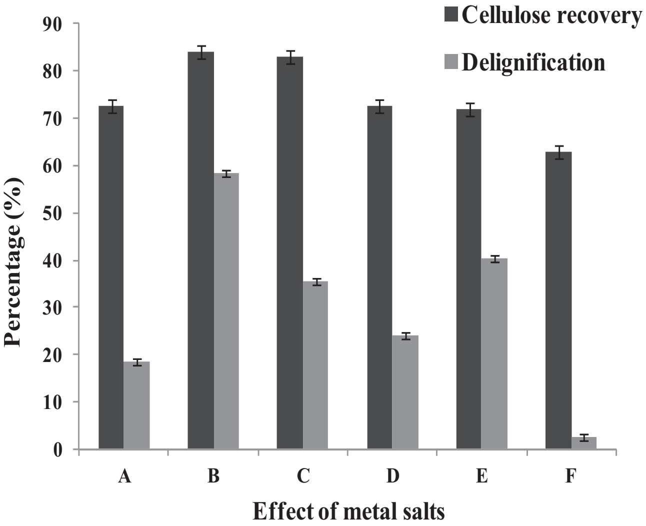

Fig. 1. Performance analysis of different metal salts with H_2O_2 for SCB pretreatment A – $H_2O_2 + MnSO_4{\cdot}H_2O$, B – $H_2O_2 + MnSO_4{\cdot}H_2O$ + ZnO, C – $H_2O_2 + MnSO_4{\cdot}H_2O + CoCl_2$, D – $H_2O_2 + MnSO_4{\cdot}H_2O + (NH_4)_6Mo_7O_{24}$, E – $H_2O_2 + MnSO_4{\cdot}H_2O + FeSO_4{\cdot}7H_2O$, F – $H_2O_2 + MnSO_4{\cdot}H_2O + TiO_2$, Conditions (1% H_2O_2, 2 g SCB, 60 min, 100 °C, 1:100 (molar ratio of metal salt and H_2O_2)).

formed during the pretreatment were analyzed and discussed in the next section. The maximum reducing sugar concentration of 21.3% was obtained from the SCB treated with $MnSO_4{\cdot}H_2O$ and ZnO.

3.2. Hydroxyl (·OH) radicals analysis

Fig. 2a and 2b show the effect of reaction time on Fe^{3+} concentration and the concentration of ions increased linearly with respect to time. The higher level of Fe^{3+} ions indicates the higher production of ·OH in the reaction mixture [20]. The maximum ·OH production and maximum dissociation of H_2O_2 was found to be 0.0646 mM/min and 216 μm/min, respectively and this was observed for $MnSO_4{\cdot}H_2O$ + ZnO system (1% H_2O_2, 2 g SCB, 60 min, 100 °C, 1:100 (molar ratio of metal salt and H_2O_2)).

3.3. Effect of hydrogen peroxide

The effect of H_2O_2 concentration on cellulose recovery and delignification is shown in Fig. 3a. The cellulose recovery increased up to 1% H_2O_2 and then decreased. The cellulose recovery and delignification observed with 1% H_2O_2 were 84.93 ± 0.84% and 59.66 ± 1.72%, respectively whereas with 2% H_2O_2, the observed values were 84.11 ± 0.34% and 60.49 ± 0.9%, respectively. The reducing sugar concentration in the liquor obtained from the pretreatment using 1 and 2% H_2O_2 was 6.2 and 8.9%, respectively. The reducing sugar formation during the pretreatment may be due to the action of metal salt, which hydrolyzed the hemicellulose part of SCB [6]. Moreover, the usage of higher concentration of H_2O_2 tends to form more inhibitory compounds and hence, 1% H_2O_2 was considered as the optimum. Takagi observed similar results with respect to treatment of newspaper with H_2O_2 [2].

3.4. Effect of substrate dosage

In order to find the optimum substrate dosage, the pretreatment of H_2O_2 with $MnSO_4{\cdot}H_2O$ and ZnO was carried out at different substrate dosages ranging from 1–4 g at optimum H_2O_2 concentration (1%). The results obtained are shown in Fig. 3b. The results showed that cellulose recovery and delignification decreased with an increase in the substrate dosage. The results showed about 88.62 ± 2.33% cellulose recovery and 69.67 ± 1.55% delignification for 1 g substrate dosage whereas for 4 g substrate dosage, cellulose recovery and delignification obtained were 54.37 ± 1.61 and 10.29 ± 0.47%, respectively. The biomass and hemicellulose recovery obtained for 1 g substrate dosage were 69.20 ± 2.38 and 47.56 ± 3.73%, respectively. The synergistic effect of H_2O_2 with the dual salts favored the maximum delignification at low substrate dosages. This may be due to the better interaction effect of hydroxyl radicals and metals with SCB at lower concentrations, which cleaved the aromatic nuclei and propyl side chains of C_α–C_β bonds of complex lignin [8].

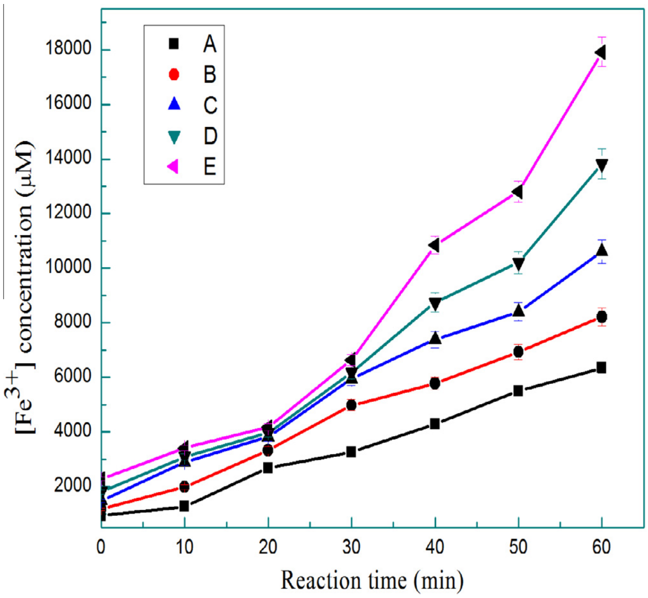

Fig. 2a. Effect of reaction time on Fe^{3+} concentration for dual salt with H_2O_2 pretreatment system. A – $H_2O_2 + MnSO_4{\cdot}H_2O + TiO_2$, B – $H_2O_2 + MnSO_4{\cdot}H_2O + (NH_4)_6Mo_7O_{24}$, C – $H_2O_2 + MnSO_4{\cdot}H_2O + CoCl_2$, D – $H_2O_2 + MnSO_4{\cdot}H_2O + FeSO_4{\cdot}7H_2O$, E – $H_2O_2 + MnSO_4{\cdot}H_2O$ + ZnO. Conditions (1% H_2O_2, 2 g SCB, 60 min, 100 °C, 1:100 (molar ratio of metal salt and H_2O_2)).

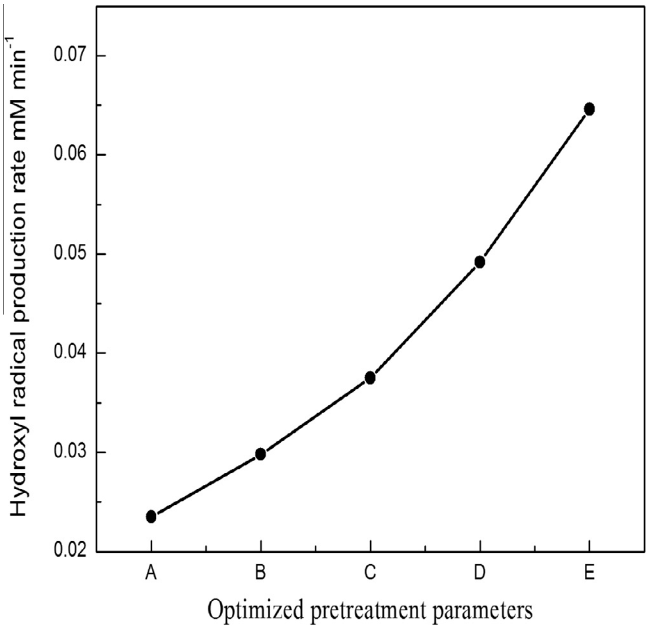

Fig. 2b. Effect of different conditions on hydroxyl radical production rate for dual salt with H_2O_2 pretreatment system. A – $H_2O_2 + MnSO_4{\cdot}H_2O + TiO_2$, B – $H_2O_2 + MnSO_4{\cdot}H_2O + (NH_4)_6Mo_7O_{24}$, C – $H_2O_2 + MnSO_4{\cdot}H_2O + CoCl_2$, D – $H_2O_2 + MnSO_4{\cdot}H_2O + FeSO_4{\cdot}7H_2O$, E – $H_2O_2 + MnSO_4{\cdot}H_2O$ + ZnO. Conditions (1% H_2O_2, 2 g SCB, 60 min, 100 °C, 1:100 (molar ratio of metal salt and H_2O_2)).

3.5. Effect of time

The effect of pretreatment time on cellulose recovery and delignification of SCB was studied and the results are shown in Fig. 3c. The reaction was carried out at optimum H_2O_2 and substrate dosage of 1% and 1 g, respectively. The cellulose recovery was increased initially with an increase in pretreatment time and then approached a constant value. The cellulose recovery obtained at the end of 15, 30, 45 and 60 min were 85.47 ± 1.87%, 89.97 ± 1.06% 89.00 ± 0.86% and 88.62 ± 2.33%, respectively. This indicates that the cellulose recovery obtained at the end of 30, 45 and 60 min of pretreatment were almost similar. The increase in reaction time from 30 to 60 min did not show significant increase in cellulose recovery but showed an increase in the production of reducing sugar. The reducing sugar concentration observed at the end of 30, 45 and 60 min were 12.85, 18.17 and 25.33%, respectively. This may be due to the depolymerization hemicellulose into simple sugars by the action of metal cations. The complex cation diffuses through SCB and protonate the oxygen of a heterocyclic ether bond between sugar monomers. Then,

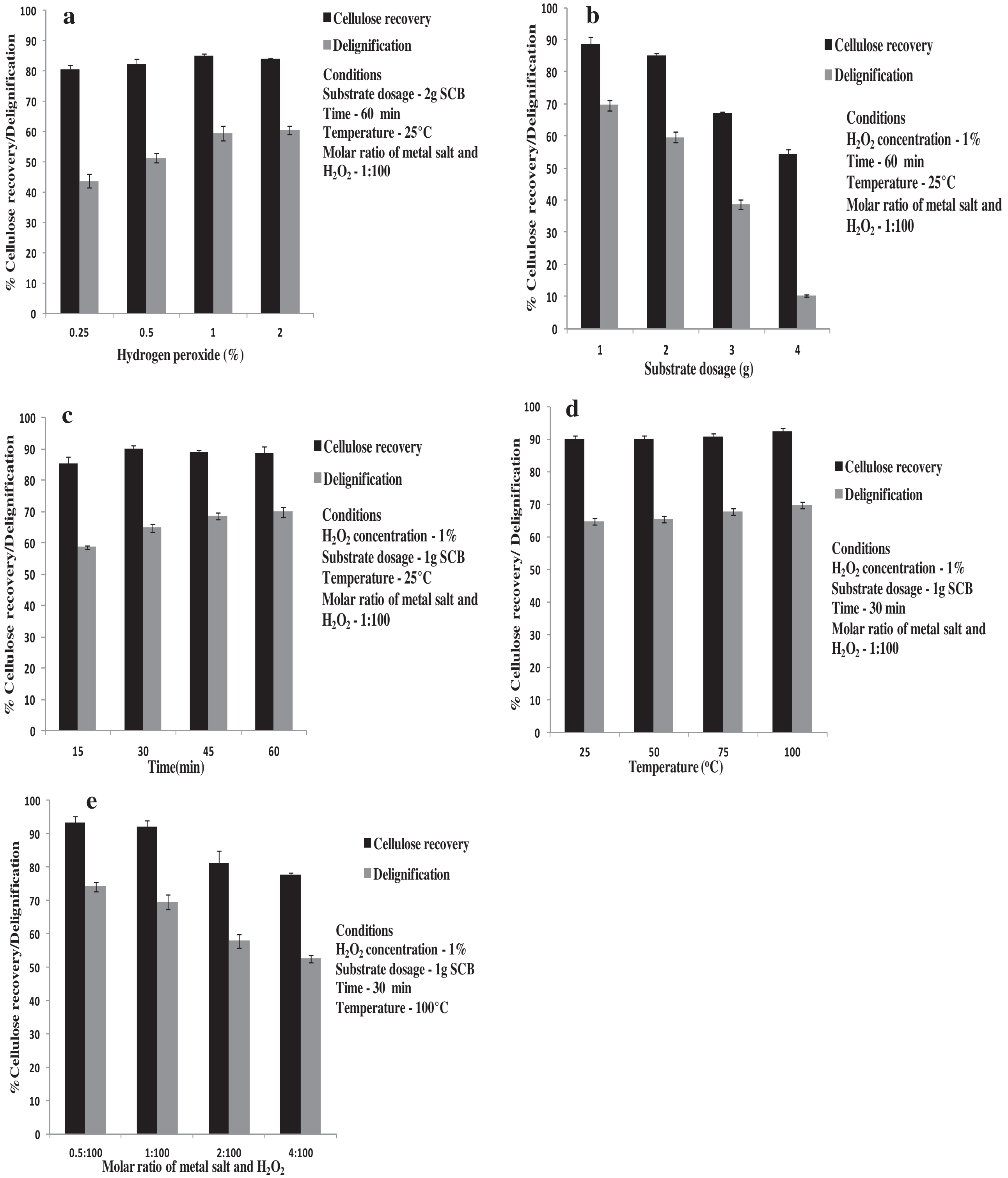

Fig. 3. Effect of different pretreatment parameters on cellulose recovery and delignification for H_2O_2 + $MnSO_4{\cdot}H_2O$ + ZnO pretreatment system.

breaking of ether bond and generation of carbocation occurs. The water molecules directly react with the carbocation through one of the two available lone pairs of electrons, resulting in a protonated hydroxide. The proton can then be abstracted by another water molecule to form a hydronium ion and the solvation of carbocation with water takes place. The regeneration of complex cation with cogeneration of sugar monomer, hemicellulose, oligomer or polymer, depending on the position of the ether bond occurs. At the end, diffusion of the reaction products in the liquid phase and re-initiation of the protonation takes place [21].

The delignification obtained at the end of 15 and 30 min were 58.69 ± 0.6 and 64.86 ± 1.2%, respectively. The delignification value increased linearly with an increase in pretreatment time and the maximum delignification of 69.87% was obtained at the end of

60 min (Fig. 3c). The recovery of cellulose is very important for the fermentable sugar production. Hence, 30 min was considered as the optimum time rather than 60 min, though the delignification value was higher for 60 min.

3.6. Effect of temperature

The pretreatment of SCB with H_2O_2 and $MnSO_4 \cdot H_2O$ + ZnO was carried out at different temperatures ranging from 25 to 100 °C at the optimum H_2O_2 and substrate dosage of 1% and 1 g, respectively. The pretreatment was carried out for 30 min. The cellulose recovery obtained at 25, 50, 75 and 100 °C were 89.97 ± 1.06, 90.18 ± 0.06, 90.69 ± 2.26 and 92.25 ± 1.67% respectively and the corresponding delignification values were 64.86 ± 1.21, 65.39 ± 1.44, 67.66 ± 0.81 and 69.67 ± 2.16%, respectively. The pretreatment temperature did not show significant effect on cellulose recovery and delignification. The results showed that operating at 25 °C would be more economical than operating at 100 °C. The hemicellulose loss observed at 25 and 100 °C was 7.5 and 41.71%, respectively. It would be beneficial if more amount of hemicellulose loss is present in the pretreatment. This is due to the fact that during dilute acid hydrolysis there would be a conversion of xylan through cyclodehydration process into furfural, which is an inhibitory product to fermentation [21]. At higher operating temperature, there might be a formation of higher amount of free radicals in the system due to breakdown of covalent bonds leading to higher reaction rates [22]. These radicals are found to actively participate in the degradation of lignin and polysaccharides [23]. The cellulose recovery and delignification value slightly increased with an increase in temperature.

3.7. Effect of metal salts

The effect of molar ratio of metal salts was investigated and the results obtained are shown in Fig. 3e. The molar ratio of metal salts to H_2O_2 was varied as 0.5:100, 1:100, 2:100 and 4:100 and the remaining parameters were maintained at their optimum values. The increase in molar ratio decreased the cellulose recovery and delignification. The results showed that 93.42 ± 1.74% of cellulose recovery and 74.18 ± 1.51% of delignification were obtained at 0.5:100 M ratio. At higher molar ratio (4:100), cellulose recovery and delignification was found to be 77.78 ± 0.52% and 52.59 ± 1.11%, respectively. The lower molar ratio was found to give maximum cellulose recovery and delignification. This may be due to the decomposition of H_2O_2 into $^{\cdot}$HO at lower molar ratio [2]. The decrease in molar ratio of metal salt to H_2O_2 (<0.5:100) did not give significant increase in the cellulose recovery and delignification. The increase in metal salt concentration reduced the quantity of oligomers in the pretreated liquid and it is due to the generation of more cations by the dissociation of metal salts, which leads to fast hydrolysis of hemicellulose [24].

3.8. Fourier-transformed infrared spectroscopy

The FTIR spectra of untreated and treated SCB are shown in Fig. 4 and this confirms the occurrence of structural changes during the pretreatment. The spectra did not show the formation of any new peaks. The cellulose is composed of β-D-glucopyranose units linked with (1 → 4) glycosidic bonds [20]. A sharp band at 900 cm^{-1} depicts the cellulose spectrum corresponding to β-glycosidic bond, between the sugar units in hemicellulose and cellulose [25,26]. The prominent band at 1029 cm^{-1} was assigned to C–O stretching vibration in cellulose, hemicelluloses, and lignin or C–O–C stretching in cellulose and hemicelluloses. The enhancement of absorption spectra at 1000–1100 cm^{-1} for the pretreated one indicates the increase in cellulose recovery in the solid residue [27]. The low intensity peak at 1156 cm^{-1} shows the presence of arobinosyl side chains [28]. The absorption band at 1238 cm^{-1} belongs to C=O stretching vibration in lignin, xylan and ester groups. The bands at 1420 cm^{-1} were attributed to the symmetric CH_2 bending and scissoring at C (6) region in cellulose [29]. The absorbance observed at 1511 and 1602 cm^{-1} in the native and

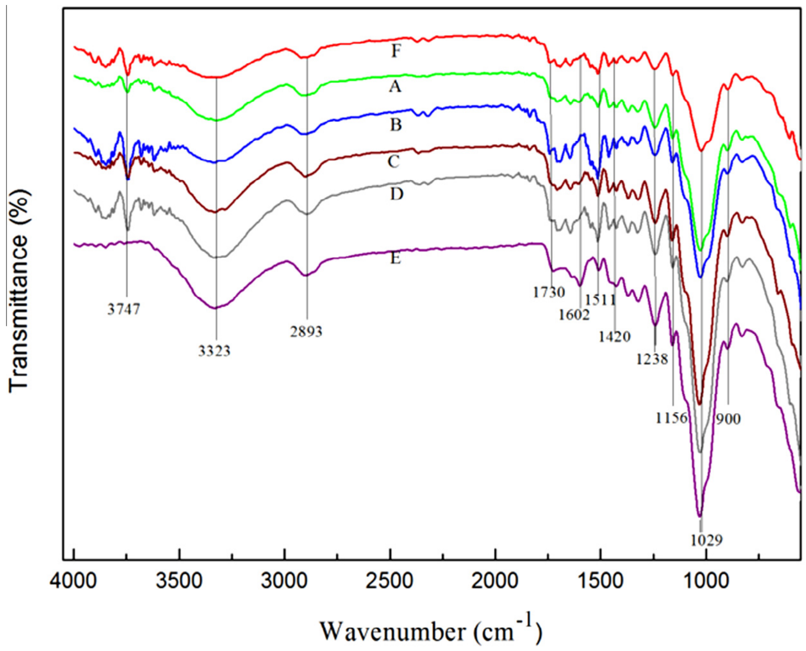

Fig. 4. FTIR spectra of SCB pretreated at different conditions for H_2O_2 + $MnSO_4 \cdot H_2O$ + ZnO pretreatment system. A – (1% H_2O_2, 2 g SCB, 60 min, 25 °C, 1:100), B – (1% H_2O_2, 1 g SCB, 60 min, 25 °C, 1:100), C – (1% H_2O_2, 1 g SCB, 30 min, 25 °C, 1:100), D – (1% H_2O_2, 1 g SCB, 30 min, 100 °C, 1:100), E – (1% H_2O_2, 1 g SCB, 30 min, 100 °C, 0.5:100), F – native SCB.

pretreated SCB, indicate the presence of aromatic skeletal vibration with C=C stretching of lignin. The band position at 1730 cm^{-1} belongs to C=O stretching vibration in acetyl groups of hemicelluloses. The broadening of the band at 3200–3400 cm^{-1} was associated with the O–H stretching of the hydrogen bonds. The absorption peak at 2893 cm^{-1} was attributed to CH_2 stretching vibration, which showed the distinguishing feature of cellulose. The sharp peak at 3747 cm^{-1} indicates the presence of an asymmetrical stretching of the O–H group.

3.9. X-ray diffraction

The X-ray diffraction profiles of the native and acid hydrolyzed SCB are shown in Fig. 5. The major diffraction peak of cellulose crystallographic plane was normally observed in the 2θ range between 21.8° and 22.1°. The crystallinity index of the native SCB was 53.28%, and it was high compared to that of the hydrolyzed SCB. The increasing order of the crystallinity index of hydrolyzed SCB was A > B > C > D > E. Similar observations were reported for the SCB pretreated using ionic liquids [30]. The crystallinity affects the hydrolysis yield and the glucose yield after the hydrolysis of ball milled SCB has been correlated with reduction in cellulose crystallinity [31]. Crystalline cellulose is very difficult to degrade, due to the strong intermolecular hydrogen bonding between the chains [32]. However, it can be degraded using acids or enzyme catalysts. The decrease in crystallinity during the acid hydrolysis may be due to the swelling of highly ordered crystalline region of cellulose present in the SCB [33]. The maximum and minimum crystallinity index [CrI] of hydrolyzed SCB was 48.56% (A) and 14.28% (E) respectively.

3.10. Scanning electron microscopy

The structural and surface morphology changes occurred due to the pretreatment was investigated. Fig. 6. shows the surface morphology of native (A) and pretreated SCB (B&C). The native SCB was smooth and continuous, and has few pores for hydrolysis, whereas the pretreated SCB has a rough surface. After the pretreatment, the rigidity of the cellulose was decreased and contorted due to the partial removal of lignin and hemicellulose present. The disruption of SCB surface becomes more pronounced, and the presence of tiny pores was clear during the pretreatment of SCB. The delignification formed pits on the cell wall structure and the surface appears more fragile, compared to untreated samples [34]. This is more suited for the acid catalyst to attack the cellulose fibers. The increase in the surface accessibility by the acid catalyst during the hydrolysis process can be attributed to the solubilization of lignin and hemicellulosic fractions. From Fig. 6, it was well evidenced that pretreatment drastically removed lignin and hemicellulosic fractions for the enhanced production of fermentable sugars.

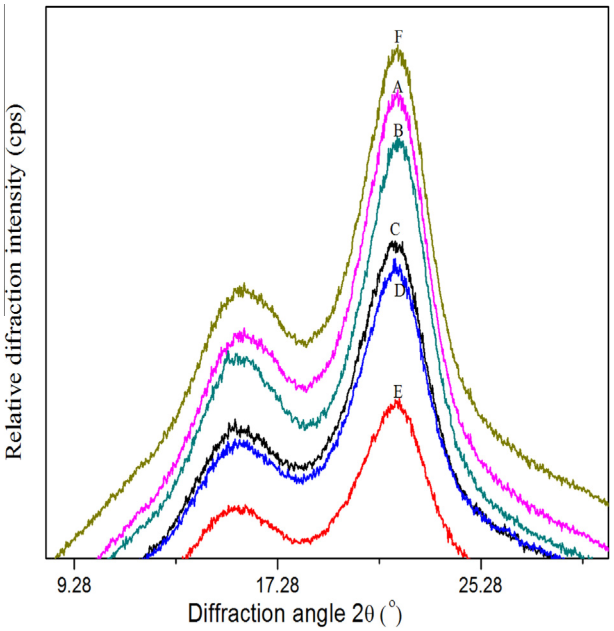

Fig. 5. X-ray crystallography of SCB pretreated at different conditions for H_2O_2 + $MnSO_4{\cdot}H_2O$ + ZnO pretreatment system. A – (1% H_2O_2, 2 g SCB, 60 min, 25 °C, 1:100), B – (1% H_2O_2, 1 g SCB, 60 min, 25 °C, 1:100), C – (1% H_2O_2, 1 g SCB, 30 min, 25 °C, 1:100), D – (1% H_2O_2, 1 g SCB, 30 min, 100 °C, 1:100), E – (1% H_2O_2, 1 g SCB, 30 min, 100 °C, 0.5:100) and F – native SCB.

3.11. Dilute acid hydrolysis

The pretreated SCB was hydrolyzed with different concentrations of dilute H_2SO_4, in order to obtain the maximum pentose and hexose sugar from SCB. The concentration of main components of SCB hydrolyzate obtained from dilute H_2SO_4 hydrolysis is shown in Table 1. It also summarizes the soluble fraction of both pentose and hexose sugars at different H_2SO_4 concentrations. The maximum concentration of xylose (11.62 ± 0.21 g/L) and arabinose (3.58 ± 0.17 g/L) was obtained when SCB was treated with 2% H_2SO_4 at 121 °C for 15 min. Under these conditions, the concentration of furfural and acetic acid were 1.19 ± 0.11 g/L and 0.68 ± 0.02, g/L, respectively. The production of acetic acid is due to the hydrolysis of acetyl groups linked between hemicellulosic fractions [35]. The inhibitor concentration decreased with the increase in the concentration of H_2SO_4, implying that higher concentration could break the pentosan more effectively with less inhibitor production. Further increase in the acid concentration (>2% H_2SO_4) was reported to decrease the sugar production, and this may be due to the increase in the release of inhibitor concentration [36]. The maximum glucose concentration of 16.85 ± 0.62 g/L was obtained at 0.25% H_2SO_4 and this is equal to the maximum theoretical yield of glucose, constituting 0.337 g/g SCB. Under these conditions, the maximum inhibitor concentration of furfural (3.07 ± 0.06 g/L) and acetic acid (1.87 ± 0.09) were observed and hence, the production of xylose (10.08 ± 0.11 g/L) and arabinose (2.05 ± 0.11) declined [14]. The present study showed the maximum pentosan concentration of 0.304 g/g SCB, which constitutes 88.97% theoretical conversion of hemicelluloses to pentose sugars. Table 2 compares the sugar yield reported in the literature with the present study and better results were observed in the present study. The maximum total sugar concentration (glucose, xylose and arabinose) of 30.46 ± 0.75 g/L was obtained with 2% H_2SO_4, whereas the minimum concentration of 28.98 ± 0.84 g/L was obtained with 0.25% H_2SO_4. There is a less significant difference in the sugar production compared to the byproduct formation throughout the acid hydrolysis, and hence, the results would be well suited for bioethanol production.

Table 3 compares the amount of inhibitor's production (acetic acid and furfural) reported in the literature with the present study and the amount of inhibitors formed was similar to that of values reported in the literature. This may be due to the substantial modifications of SCB during the metal salt pretreatment with H_2O_2. Furthermore, the novel pretreatment results in higher catalytic efficiency [E] value of 10.6% with 2% H_2SO_4, and the lowest [E] value of 6.49% with 0.25% H_2SO_4. Hence, this pretreatment method significantly facilitated the higher fermentable sugar production.

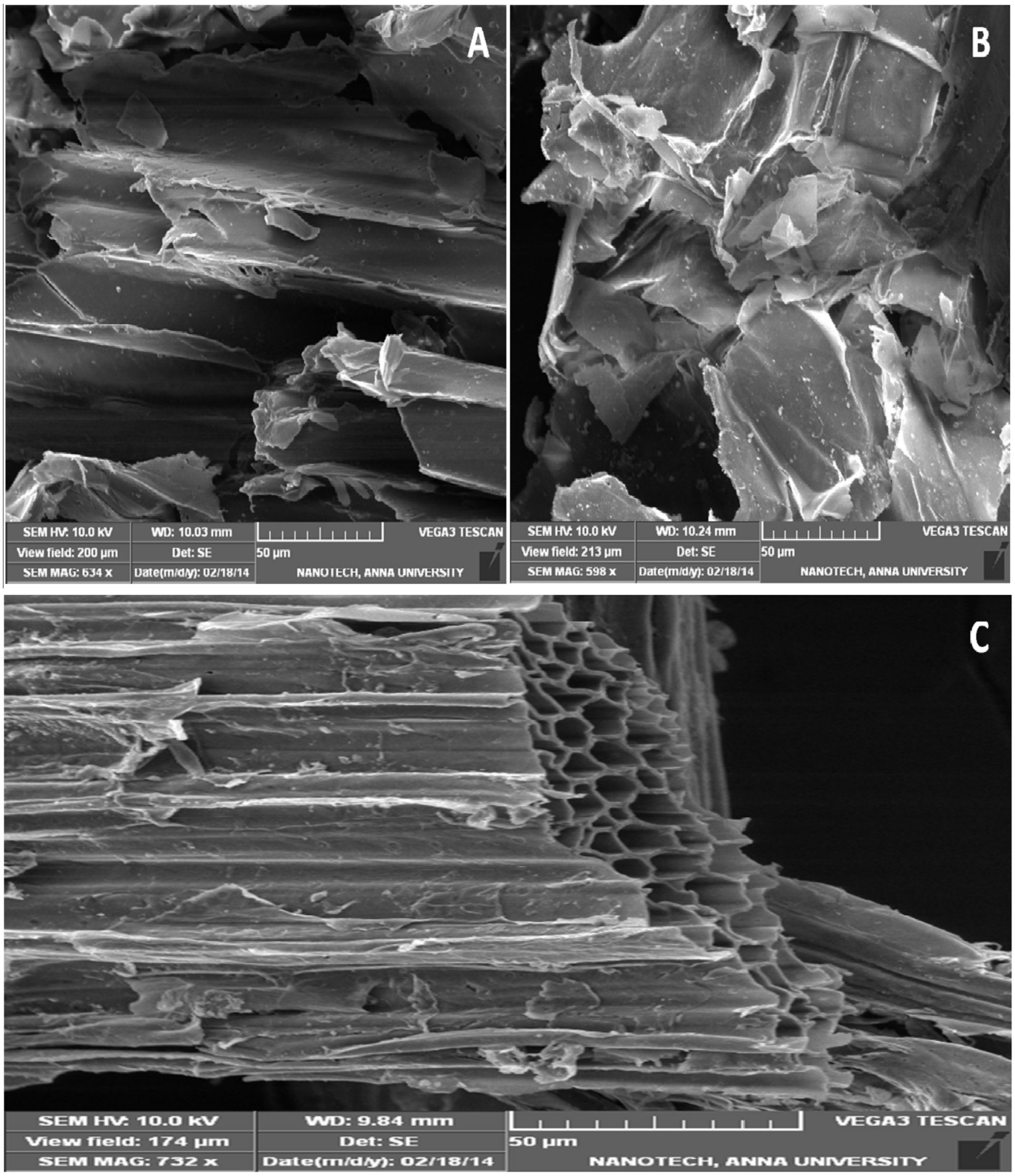

Fig. 6. Scanning electron microscopy images of SCB fibers (A) Native SCB (B) SCB pretreated with H_2O_2 and metal salts ($MnSO_4$ and ZnO) (C) SCB pretreated with H_2O_2 and metal salts ($MnSO_4$ and ZnO) (cross sectional view).

Table 1
Dilute acid hydrolysis profile of pretreated SCB and its hydrolyzed composition.

Temperature (°C)	Reaction time (min)	H_2SO_4 (%, v/v)	Xylose (g/L)	Glucose (g/L)	Arabinose (g/L)	Acetic acid (g/L)	Furfural (g/L)
121	15	0.25	10.08 ± 0.11	16.85 ± 0.62	2.05 ± 0.11	1.87 ± 0.09	3.07 ± 0.06
121	15	0.5	10.76 ± 0.08	16.05 ± 0.58	2.56 ± 0.21	1.36 ± 0.12	2.39 ± 0.05
121	15	1	11.10 ± 0.39	15.81 ± 0.13	3.07 ± 0.10	1.02 ± 0.18	1.87 ± 0.36
121	15	2	11.62 ± 0.21	15.26 ± 0.37	3.58 ± 0.17	0.68 ± 0.02	1.19 ± 0.11

3.12. Fermentation

The SCB hydrolyzate containing 15.26 ± 0.62 g/L of glucose, 11.62 ± 0.21 g/L of xylose and 3.58 ± 0.17 g/L of arabinose was fermented using *S. cerevisiae* and the ethanol production observed at the end of 72 h was 13.1 g/L and this corresponds to 84.32% theoretical yield. In this study, the ethanol production was found to be 0.429 g/g sugar with a yield of 0.184 g/g of SCB.

4. Conclusions

This study presented the influence of dual salt on the pretreatment of SCB with H_2O_2. Among the different dual metal salts studied, $MnSO_4{\cdot}H_2O$ and ZnO showed better performance and $MnSO_4{\cdot}H_2O$ and TiO_2 showed least performance. The different operating parameters such as H_2O_2 concentration, SCB dosage, reaction time, temperature and molar ratio of metal salts to H_2O_2

Table 2
Comparison of sugar yield reported for various hydrolysis conditions of SCB with present study.

Feedstock	Pretreatment	Hydrolysis conditions	Total sugar (g/g biomass)	Refs.
SCB	Ammonium hydroxide pretreatment	0.5% sulfuric acid, 110° C,4 h, LSR 1:15 g/mL	0.162 g/g	[37]
SCB	–	2% sulfuric acid, 122 °C, 24 h, LSR 1:10 g/mL	0.246 g/g	[38]
SCB	–	0.045 M sulfuric acid, 170 °C,10 min	0.251 g/g	[39]
SCB	–	0.5% sulfuric acid,121 °C, 60 min, LSR 1:15 g/mL	0.367 g/g	[40]
SCB	Ultrasound assisted ammonia pretreatment	2% sulfuric acid, 45 min, LSR 1:20 g/mL	0.49 g/g	[14]
SCB	Ultrasound assisted alkali pretreatment	2% sulfuric acid, 45 min, LSR 1:20 g/mL	0.58 g/g	[35]
SCB	H_2O_2 with bimetallic salts	2% sulfuric acid, 15 min, 121 °C, LSR 1:20 g/mL	0.609 g/g	Present study

Table 3
Comparison of inhibitors production during hydrolysis of SCB and their catalytic efficiency.

Feedstock	Hydrolysis condition	Inhibitors			Catalytic efficiency (E)	Refs.
		Furfural (g/L)	Acetic acid (g/L)	HMF (g/L)		
SCB	2% sulfuric acid, 50 °C, 45 min, 1:20 g/mL	0.59	1	ND	9.32	[14]
SCB	1.25% v/v sulfuric acid, 121 °C for 120 min	1.4	4.0	ND	4.11	[41]
SCB	0.045 M sulfuric acid, 170 °C for 10 min	1.43	2.72	0.11	4.77	[39]
SCB	0.5% HCl, 100 °C for 5 h	1.19	0.15	ND	10.85	[37]
SCB	2% sulfuric acid, 15 min, 121 °C, LSR 1:20 g/mL	1.19	0.68	ND	10.6	Present study

were optimized and their respective optimum values were found to be 1% H_2O_2, 1 g SCB, 30 min, 100 °C and 0.5:100 g/mL. The maximum catalytic efficiency [E] and sugar yield of 10.6% and 30.46 g/L were obtained at the optimum hydrolysis conditions of 2% H_2SO_4, 15 min, 121 °C and LSR of 1:20 g/mL. The fermentation of hydrolysate produced 13.1 g/L of ethanol and this corresponds to 84.3% of theoretical yield. The yield of ethanol obtained at the end 72 h was 0.184 g/g SCB. In conclusion, the production of sugars from SCB by dual salt pretreatment appears to be attractive.

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